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(54) Histones for use in therapeutic procedures.

(57) The invention relates to the use of pure histones H1, or the histone dimer H2A:H2B for therapeutic procedures. Instead of the pure histones active segments thereof with hormone or hormone like activity especially thymic hormone like activity can be used alone or in combination with the pure histones.

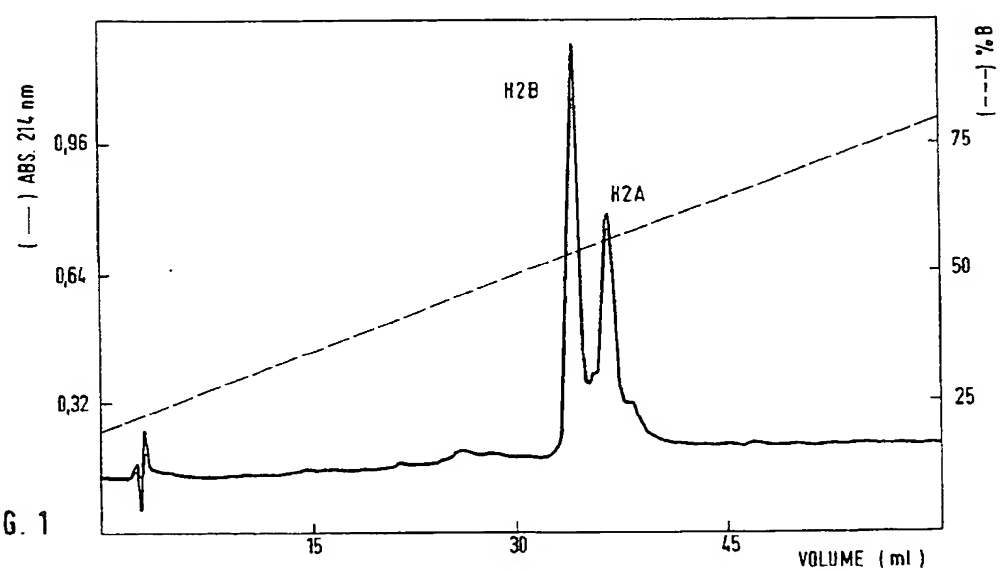

FIG. 1

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HISTONES FOR USE IN THERAPEUTIC PROCEDURES

According to the present state of knowledge histones H1, H2A, H2B, H3 and H4 are the essential components to the cell nucleic and are together with DNA the substantial components of chromatin.

In the European Patent 0 149 468 it is proposed pure histone H2A and/or H2B or H3 or active segments thereof (the evolutionary variable part or partial sequences of this part or the N-terminal part). It is nothing said about histone H1 and the histone dimer H2A:H2B. The histones have hormonal or hormone-like and immuno-stimulating functions. Such functions so far have never been brought in connection with histones. For the experts it was novel and surprising that the histones are freely moving in the blood circulation and the lymphatic system of healthy organisms and will bring about a biological response due to the interaction with specific receptors of living cells. There is provided the use of histone for stimulating the immune system, for the therapy of the immune system, for the treatments of immune deficiency, of the consequences of the thymectomy and the following of massive irradiation of the thymus, for the damping of thyroxine, desoxycorticosterone, thyrotropin, gonadotropin and for the strength of growth hormone.

In U.S. Patent 4 451 553 it is proposed to prepare an antigen from human or animal cancer cells, which is injected to persons or animals suffering from cancer in order to incite the production of antibodies, which are not only intended to destroy the antigens but also the very own cancer cells. From the cancer cells a malignant total histone is being isolated, which is not divided into the individual histones, and the total histone is bonded to malignant DNA and RNA in order to form the antigen.

In "Chemical Abstrats" 72, 109724 (1970) it was proposed to isolate fragments of the histones which are rich with arginine and lysine, to charge these fragments with reactive groups and to take advantage of their bondage to nucleic acids for chemically changing the nucleic acids by means of said reactive groups. Said literature discloses the idea of using specific histone fragments as a carrier for chemotherapeutic substances applied in the treatment of cancer.

In "Chemical Abstracts" 74, 85743 (1971) it is said that the resulting total histone fraction will prevent the formation of antibodies against T_2-bacteriophages if given simultaneously with the antigen (the phage) and furthermore in very large doses (50 mg/kg).

In "Chemical Abstracts" 73, 96837 (1970) the immuno-suppressive effect of total histone is described by the example of a human skin transplantation.

The above mentioned state of the art neither discloses nor suggests the discovery of the invention that pure histone H1 and the pure histone dimer H2A:H2B may be used in therapeutic procedures.

It is the problem of the invention to provide histones or histon complexes other then H2A, H2B, H3 for specific therapeutic purposes and to provide the use of histones in new therapeutic procedures.

The problem is solved by using the pure histone H1 together with the subtyps or the histone dimer H2A:H2B, or parts thereof with hormonal or hormone-like activity especially thymic hormone like activity for the preparation of pharmaceuticals used in immunotherapy, the therapy of endocrine disturbances and in cancer therapy. Further the invention provides the histones and there active segments for the treatment of carcinoma (melanoma, sarcoma, mesothelioma, malignant deceases, especially those of the lymphatic system originating from malignant B- and T-cells such as B-lymphoblastic lymphoma, myelogenic leukemia, Burkitt lymphoma).

It is an advantage of the pharmaceutical according to the invention that it can be used for the treatment of cancer and radiation induced leukemia, AIDS-diseases, for dampening the function of the suprarenal cortex, the thyroid, of the gonads, for supporting the function of the hypophysis and for the treatment of after-effects resulting from the removal of the thymus gland (thymectomy).

In the following examples the therapeutic efficiency of the pharmaceutical according to the invention is shown. The active substance of the examples 1 to 4 is the histone dimer H2A:H2B according to Fig. 1.

The active substance H2A:H2B according to the invention (Fig. 1) was prepared by the inventors from a thymus preparation (homeostatic thymus hormone) from calf thymus according to Comsa & Bernardi ("Extraction Fractionation and Testing of Homogenous Thymic Hormone Preparation", Anals of the New York Academy of Sciences, Vol. 240, pages 402-403, Feb. 28, 1975) by means of high speed liquid chromatography. Further details will follow in experiment 1. In the same manner pure H2A and pure H2B can be prepared. The active substance H2A:H2B (Fig. 1) is received by a mixture of 1:1 of pure histones H2A and H2B.

The invention is not restricted to the pure dimer H2A:H2B, but also extends to the pure dimer together with pure H2A and/or pure H2B or parts thereof with hormone or hormone like activity especially thymic hormone like activity.

As shown by the following experiments the inventors were able to prove that the pure histone H1 and

the pure histone dimer H2A:H2B can be used for cancer therapy. But the histones are not only suited for cancer therapy, in particular the treatment of malginant cancer of lymphoma cells, but also for the immunotherapy (e.g. AIDS-diseases) and for the therapy of endocrine disturbances.

The concentrations of H2A:H2B to be given will be between 50 and 500 µg/l. They will be given especially by subcutane or intramuscular injection. Said injections preferably are physiological salines with may be phosphate-buffered and which contain H2A and/or H2B or their active components.

Example 1

The efficiency of H2A:H2B was tested in vitro with myeloma cells (cancer cell line P3Ag8.653, Flow Laboratories) of the mice inbred strain BALB/c. Said subklon of the foregoing cancer cell line adheres to the bottom of petri dishes because of long-term cultivation and selection.

There are no immunoglobulines preticipated, it does not grow in HAT-medium and easily amalgamates with spleen cells.

The cultivation of the cancer cells took place in a culture medium of:

RPMI 1640 with L-glutamine without $NaHCO_3$	10.39 g/l
Penicilin G	2×10^4 int. units
Streptomycin sulfate	2×10^4 int. units
L-Glutamine	1 % 200 mM
Pyruvate	1 % 200 mM
$NaHCO_3$	0.2 %
β-Mercaptoethanol	1 % 5×10^{-3} M
Fetal calf serum (= FCS) of Biochem, Berlin	10 %
pH-value	7.2

The H2A:H2B used was prepared from a purified histone preparation from calf thymus by means of high speed liquid chromatography (Fig. 1). The elution was carried out at a µBondapak C-18-column with a linear gradient (%B) of 20 to 80 % acetonitrile in 0.1 % trifluoracetic acid with a flow of 1 ml/min. The adsorption was measured at 214 nm. In Fig. 1 the abscissa shows the volume in ml, the left ordinate shows the absorption at 214 nm and the right ordinate shows the linear gradient (%B).

The cells are fed with completed culture medium (RPMI 1640 with 10 % FCS). The culture medium was renewed each day. When the bottom of the culture dish was fully overgrown, the cells were scraped off and part of them was transplanted, as for the tests cells in their optimum environment for growth were needed. Breeding was performed at 36.5° C and 5.5 CO_2 in an incubator.

The concentration of viable cells was determined with the coloring agent Nigrosin (0.2 % in phosphate-buffered saline (PBS)) in the Neubauer-counting chamber.

Completed culture medium was added to freeze-dried H2A:H2B concentration (storage at -20° C) and filtered under sterile conditions. (Sterivex-Filter System GV of Millipore, Munich). With 400 µg/ml the concentration of H2A:H2B was twice as high as the highest concentration needed for the experiment. For lower concentrations the solution was diluted accordingly.

For the experiment cells were scraped off from the bottom of not too thickly grown culture dishes, the number of living cells was determined and adjusted to 3.5×10^5 cells/ml. 100 µl H2A:H2B solution or a thinner were added to 100 µl of said cell suspension and incubated in an incubator. The final concentration of the cells per dish was 1.75×10^5 cell/ml with a concentration of H2A:H2B of 200, 150, 125, 100 and 50 µg/ml. The experiment was carried out for 6 days, the viable cells were counted once a day, in the first two days twice a day. For each concentration therefore eight starting preparations were needed.

In Fig. 2 the viable cell concentration (= viable cell number/ml) and the vitality are graphically compared to time (period of incubation). The vitality relates to the originally used cell concentration (1.74×10^5 cells/ml = 100 %):

$$\text{vitality (\%)} = 100 \times \frac{\text{viable cell concentration}}{1.74 \times 10^5 \text{ cells/ml}}$$

In all concentrations H2A:H2B shows a cytotoxic effect to the tested cancer cell line, the efficiency, however, is different.

In Fig. 2 the timely course of the vitality and the vaible cell number/ml of the cancer cells on incubation with different concentrations of H2A:H2B is shown graphically. Concentrations up to 150 µg/ml of H2A:H2B did not show any or only an inferior retardation of the cell growth. With a concentration of 200 µg/ml of H2A:H2B the killing rate was 80 % at the first day. Subsequently the cells continued growing like in the other concentrations used and like in the check concentration. The initial number was reached after about three days.

Example 2

The efficiency of H2A:H2B was furthermore tested in vitro at a human cancer cell line. It was the cancer cell line IM-9 from the marrow of a female patient with multiple myeloma which was used. For B-cells said cancer cell line has receptor positions for human growth hormones, for insulin and calcitonin.

Description and origin of the cancer cells:

Growth properties: 4-5-fold doubling within 5-7 days with an initial number of cells of 3-4 x 10^5 of viable cells/ml
Morphology: Lymphoblast-like
Karyology: Chromosome frequence distribution 50 cells
2/43; 6/45; 36/46; 5/47; 1/48 2n = 46
human, female, stemline number = 46;
dipoid, stable Karyotype
Sterility: Test for mycoplasma, bacteria and fungi was negative
Reverse transcriptase: negative
Surface immunoglobulines: detected
EBNA: positive
Submitted: D.N. Buell, NIH, Bethesda, Maryland USA
Prepared and characterized: American Type Culture Collection, Rockville, Maryland
Literature: Buell D.N., (1972) Ann. N.Y. Acad. Sci. 190, 221-234;
Proc. Natl. Sci. USA 71, 84-88 (1974);
J. Biol. Chem. 249, 1661-1667 (1974).

For control freshly isolated spleen cells from mice inbred strains BALB/c were used. The cells were kept in culture under sterile conditions at 37° C, 5.5 % CO_2 and 95 % of relative humidity. As medium RPMI 1640 with 10 % fetal calf serum of Biochrom, Berlin, was used.

To the culture medium (according to experiment 1) H2A:H2B (according to experiment 1) with a final concentration of 180 µg/ml was added. 2 x 10^5 viable cells were cultivated in said medium and the total cell number and the number of viable cells were counted daily. For this purpose one part of a solution of 0.1 mg acridine orange and 0.1 mg ethidium bromide in 100 ml phosphate buffered physiologic saline (PBS) were mixed with an equal volume of cell suspension and evaluated immediately under a fluorescence microscope. Living cells take up acridine orange and appear green when a blue excitation is used, whereas ethidium bromide diffuses into dead or dying cells and turns the cells into orange. When a green excitation is used, living cells are not turned into color, whereas dead cells appear red.

H2A:H2B originates form a purified histone preparation according to experiment 1.

The timely course of the vitality and/or the viable cell number/ml of the human tumor cell line IM-9 after a single dose of 180 µg H2A:H2B/ml cell suspension is shown in Fig. 3. The viable cell number was determined under a fluorescence microscope by means of the vital coloring agents acridin-orange and ethidium bromide.

Already after a single dose of 180 µg of the dimer is given to a cell suspension with 2 x 10^5 cells/ml the number of viable cells reduced by more than 80 % within 3 days. This reduction was continued with more than 90 % of killed cells up to the 6th day of the experiment. Within the test period of 7 days no growth of the rest of the surviving cells was observed, which corresponds to a complete breakdown of the cancer cells. The number of untreated cancer cells has become fourfold as high as in the beginning of the experiment. Not degenerated spleen cells treated with 180 µg HTH/ml (2 x 10^5/ml) showed a nonsignificant higher death rate in the same period of time.

After a single dose of H2A:H2B a selective killing of the human cancer cell line IM-9 follows. With a dose of 180 µg/ml of medium the human multiple lyelom line with a cell density of 2 x 10^5/ml has a complete breakdown, whereas the normal spleen cells from BALB/c mice only show an insignificant increase in the natural death rate.

In the following examples the therapeutic efficiency of the pharmaceutical according to the invention for cancer therapy is shown. The active substance is a H2A:H2B histone mixture or histone complex according to Fig. 1. It is very likely that positive effects can also be achieved with a histone H2A alone or with a histone H2B alone.

The comparison of the sequences with known thymic hormons shows that the hormonal or hormon-like acticve components of the histones are their evolutionary variable sections. Therefore it is probable that an effect according to the invention can also be achieved with at least one evolutionary variable histone section of the histones H2A and H2B. It is furthermore known that at least a partial effect may be achieved already when a partial section of at least five amino acid residues is present. Therefore it can be assumed that an effect according to the invention can already be achieved with one partial section of at least five aminoacid residues of at least one evolutionary variable histone section of the histones H2A and/or H2B.

This makes it evident that H2A:H2B does not only retard the growth of malignant lymphom cells but can entirely kill the cells. Comparable successes so far have not been achieved with the cytostatics, such as cyclophosphamide, as presently used for chemotherapeutics, which do also kill normal, not degenerated cells and cause heavy secondary effects in clinical use. While H2A:H2B in a concentration of 180 µg/ml makes completely disappear malignant lymphom cells : healthy lymphatic cells are only insignificantly influenced in their growth.

If H2A:H2B can kill lymphom cells, then also other kinds of cancer cells, as e.g. carcinomes, melanomes, andenomes should be treatable with H2A:H2B, whereby the growth retardations in specific periods and with specific doses will probably be different.

Example 3

Differential effect of histone dimer H2A:H2B on plasma thyrotropin and growth hormone in young and old rats

The histone H2A:H2B dimer reduces plasma thyrotropin (TSH) and growth hormone (GH) in young (3mo) Sprague-Dawley male rats, but fails to do so (TSH) or has a significantly weaker effect (GH) in old (26 mo) animals. Young and old conscious, free-moving rats carrying an indwelling atrial cannula received the substances to be tested via the cannulas. Plasma samples were taken every 30 min for 5 h and hormones were measured by RIA. In the young rats histone H2A:H2B (8 mg/kg B.W.) induced a marked reduction in plasma TSH which was significantly greater than the normal circadian decline observed in saline-injected young controls. The old rats displayed high basal levels of TSH which showed no circadian rhythmicity and did not respond to histones H2A and H2B. Plasma thyroxin (T_4) showed a significant age-related reduction but was not affected by histones. The above dose of H2A:H2B significantly reduced plasma GH in both young and old rats, but the effect was greater in the young animals. Mean basal levels of plasma GH were significantly lower in old than in young rats. The present results suggest that histones H2A and H2B, whose production by the thymus is known to be stimulated by TSH and GH, is involved in an inhibitory feedback loop regulating plasma TSH and GH in young rats. The present data also add to the growing evidence that the immune and the neuroendocrine systems function coordinately. The results additionally suggest that a disruption in immune-neuroendocrine integration occurs during aging. This disruption could play a significant role in the age-associated immunopathologies that occur in both humans and laboratory animals (Fig. 4 and 5).

Example 4

Histone H2A:H2B dimer increases plasma levels of corticosterone but not prolactin (Prl) in a dose- and age-dependent manner in male Sprague-Dawley rats. Young (3 mo) and old (26 mo) conscious free-moving animals carrying an indwelling atrial cannula received the substances to be tested via the cannulas. Plasma samples were taken every 30 min. for 5 h and hormones were measured by RIA. Histone doses of 1 and 8 mg/kg B.W. injected into young rats elicited a 7,8- and 12,8-fold increase in plasma corticosterone,

respectively, as compared to saline-injected controls. The histone-induced peak corticosterone levels were reached within 1.5 and 2,5 h after 2,5 h after H2A:H2B injection. Plasma Prl was not affected by the histones in either age group. A single dose of 8 mg H2A:H2B/kg B.W. induced a smaller corticosterone response in old than in young rats. Although the time course of the response was similar in both age-groups. Depending on the now known activities of histones H2A and H2B the present results add to the growing evidence that the immune and neuroendocrine systems function coordinately. The data also suggest that a disruption in immune-neuroendocrine integration occurs during aging. This disruption could play a significant role in age-associated immuno-pathologies, particularly autoimmunity (Fig. 6).

Preparation of H1 Histones

Thymus glands from calf were commercially obtained by slaughter houses. Glands taken directly from animals were immediately frozen on dry ice and stored at -30° C.

Whole glands were cut to pieces and homogenized in three volumes of 0,3M sucrose containing 3 mM $CaCl_2$. The homogenate was centrifuged for 10 minutes at 200g. The combined crude pellets were homogenized in 10 volumes of 2,5 M sucrose, 3 mM $CaCl_2$ and centrifuged for 60 min at 45 000 g. The pellets containing the nuclei were combined and washed twice with 0,5M sucrose, 3 mM $CaCl_2$ and once with 0,15M NaCl, 10 mM EDTA. After each step of washing the pellets were centrifuged at 1000 g for 10 min.

Total histones (H1, H2A, H2B, H3, H4) were extracted from the combined nuclear pellets by treatment with 0,4 N H_2SO_4 at 0° C for 15 h. The mixture was centrifuged at 70 000g for 30 min, 5 vol. of cooled ethanol was added to the supernatant, and the mixture was stood overnight at -20° C. H1 histones were extracted from the mixture with 5% perchloric acid for 3h, and the mixture was centrifuged at 200g for 10 min subsequently. The supernatant was dialyzed against 1mM acetic acid overnight. H1 histone was precipitated by the addition of 100 % trichloroacetic acid (1 gm/ml) to a final concentration of 20% at 0° C. After 15 min the precipitate was collected by centrifugation at 3 500 g for 15 min and washed once with acidified acetone (0,5 ml conc. HCl/100ml) and twice with acetone. After centrifugation at 500 g for 10 min the pellets were soluted in 1 mM acetic acid and lyophilized to obtain H1 histones.

The action of Histone H1 was tested in vitro on six human cancer cell lines originating from B-cells of the lymphatic system. The concentration of H1 tested was 250 mcg/ml medium for the following cancers strains: Daudi, EB 2, CCRF SB, CCRF CEM and Namalwa and 180 mcg/ml for the IM9 strain. A maximal decay of cancer cells was observed between the 3rd and 6th day and reached up to 100%. 4 cancer cell lines began to grow again, but did not reach the initial cell concentration. The treatment of normal spleen cells of mice showed that H 1 had no significant toxicity on these cells at a concentration of 50-200 mcg/ml under the conditions employed.

The following cell lines has been used:

DAUDI: Burkitt Lymphoma of a 16 year old negro. Translocation of parts of the chromosomes 8 and 14 led to the activation of an oncogene. There are binding sites for the fc-receptor and for receptors for complement and immunoglobulins.

EB2: Burkitt Lymphoma of a 7 year old negro. The line contains free active Epstein-Barr virus particles.

IM9: Myelogenic Leucemia of a female patient. The cell line is able to produce IgG. There are receptors for human growth factors, insulin and calcitonin.

CCRF SB: B-lymphoblatic Lymphoma of a 11 year old caucasic girl. The line is not able to produce IgG.

CCRF CEM: B-lymphoblastic Lymphoma of a 4 year old caucasic boy. The line is able to produce IgG. No common receptors are B-cells on the membranes.

Namalwa: Burkitt Lymphoma

The tested cell lines were obtained from FLOW Lab. and grew in RPMI-1640 medium with 10% FCS in a humified incubator with 5,5% CO_2. $2x10^5$ cells/ml medium were incubated with 180- 250 mcg hormone/ml and spread in 96 well dishes. The viable cells were counted by the ethidium bromide and acridine orange method (EB/AO).

Example 5

DAUDI showed a 96 % decay of cells after two days and had 20 % viable cells at the end of the experiment (Fig. 7).

Example 6

EB2 showed a continuous dying of cells up to the 6th day and had 70 % vital cells at last (Fig. 8).

Example 7

IM9 cells were very sensitive and showed a survival rate below 10 % within 3 days. After 6 days no living cells were observed (Fig. 9).

Example 8

CCRF SB: A maximal death rate of 58 % of the cells was observed on the 3rd day. On the 6th day the cells grew up to 80 % of the initial cell concentration (Fig. 10).

Example 9:

CCRF CEM: On the 5th day about 95 % of the cells were killed and on the 6th day a sligth growth had occured to about 20 % of the initial cell conentration (Fig. 11)

Example 10

Namalwa was also very sensitive against H1 and showed a maximal decay rate of 99 % after 4 days. Little growth occured on day 5 and day 6 (2 % of the initial cell concentration) (Fig. 12)

To summarize, pure Histone H1 was cytoxic against all tested human cancer cells when it was applied as a single dose at a concentration of 180/250 mcg/ml. The cell line IM9 was the most sensitive one and was completely killed within 6 days. Three of the tested cell lines were Burkitt Lymphomas (Daudi, Namalwa & EB2). While more than 95% of the Daudi and Namalwa cells were killed, EB2 cells represent the most resistant cell line (death rate 30%). It is possible that this behaviour depends on the presence of EB-Virus particles. Both CCRF SB and CCRF CEM showed approximately 50% death rate on the 3rd day. While the number of living cells of CCRF CEM was about 5% on day 5, the cells of CCRF SB were growing again from day 2 and reached about 80% of living cells in compared to the initial cell concentration (Fig. 13).

Fig. 14 shows the viability of normal murine spleen cells as function of H1-concentration from 50 µg/ml to 200 µg/ml.

Example 11

Concerning lymphoma:

The cell line OH was obtained from a patient of the University Hospital of Lund, Department of Lund Medicine, and cultered as described in Example 1 except the cell density which was adjusted to 315 cell per se. Incubation with a single dose of 200 mg H1 Histone almost completely arrested the growth of the cells, whereas the control grew from 3 x 10^5 cells perml to 1,2 x 10^6 cells per ml within six days (Fig. 15;-(OH200))

Example 12

Concerning melanoma:

The cell line E6 was obtained from the University Hospital of Lund and cultivated as described in

Example 1. The first day of incubation with a single dose of 200 mg per ml of histone H1 the cell density was determined to 40 x 10^4 cells per ml. In the sample 1 treated with histone H1 the cell number stayed roughly constant until day four and increased to 90 x 10^4 cells per ml at day 6. In the control the number of cells increased to 90 x 10^4 cells per ml at day 3 and to 140 x 10^4 cells per ml at day 6 (Fig. 16; (EG 200)).

Example 13

Concerning sarcoma:

The cell line BAW was obtained from a patient of the University Hospital at Lund and cultivated as described in Example 1. The cell density was determined to 6 x 10^4 cells per ml in the experiment and to 7 x 10^4 viable cells per ml in the control immediately upon incubation with 200 mg per ml histone H1. As shown in Fig. 17 (BAW 200) after day 5 the number of viable cells had decreased below 5 x 10^4 cells per ml whereas the untreated control had increased to about 17 x 10^4 cells per ml.

Claims

1. Pure histone H1 including all subtyps or active segments thereof with hormone or hormone like activity especially thymic hormone like activity for use in the therapeutic procedures.

2. Pure histone dimer H2A:H2B or an active segment thereof with hormone or hormone like activity especially thymic hormone like activity for use in therapeutic procedures.

3. A histone according to claim 2 together with pure histone H2A and/or H2B or at least one active segment thereof with hormone or hormone like activity especially thymus hormone like activity.

4. A histone according to one of the claims 1 to 3 for the immunotherapy, the therapy of endocrine malfunctions and cancer therapy.

5. A histone according to one of the claims 1 to 3 for the treatment of the consequences of the thymectomy or the following massive irradiation of the thymus.

6. A histone according to one of the claims 1 to 3 for the treatment of radiation-induced leukemia or carcinoma.

7. A histone according to one of the claims 1 to 3 for the damping of the thyroxine or ACTH or desoxycorticosterone or thyrotropin or gonadotropin.

8. A histone according to one of the claims for the treatment of the strengthen of groth hormone.

9. A histone according to claim 4 for the treatment of carcinoma (melanoma, sarcoma, mesothelioma and malignant diseases, especially, those of the lymphatic system originating from malignant B- and T-cells such as B-lymphoblastic lymphoma, myelogenic, leukemia, Burkitt-lymphoma).

10. A histon according to one of the claims 1 to 3 extracted from an endocrine gland of an animal especially from the thymus gland of a calf.

11. A histone according to one of the claims 1 to 10 wherein the pure histones or its active segments are in connection with at least one protein of the immune system.

12. A histone according to one of the claims 1 to 11 wherein the pure histones or its active segments are in connection with ubiquitin.

13. At least one biologically active pure histone selected from the group consisting of H1, H2A, H2B, dimer H2A:H2B, H3, segment thereof with hormone or hormone like activity especially thymic hormone like activity for the treatment of carcinoma (melanoma, sarcoma, mesothelioma, malginant diseases, especially those of the lymphatic system originating from malignant B- and T-cells such as B-lymphoblastic lymphoma, myelogenic leukemia, Burkitt-lymphoma).

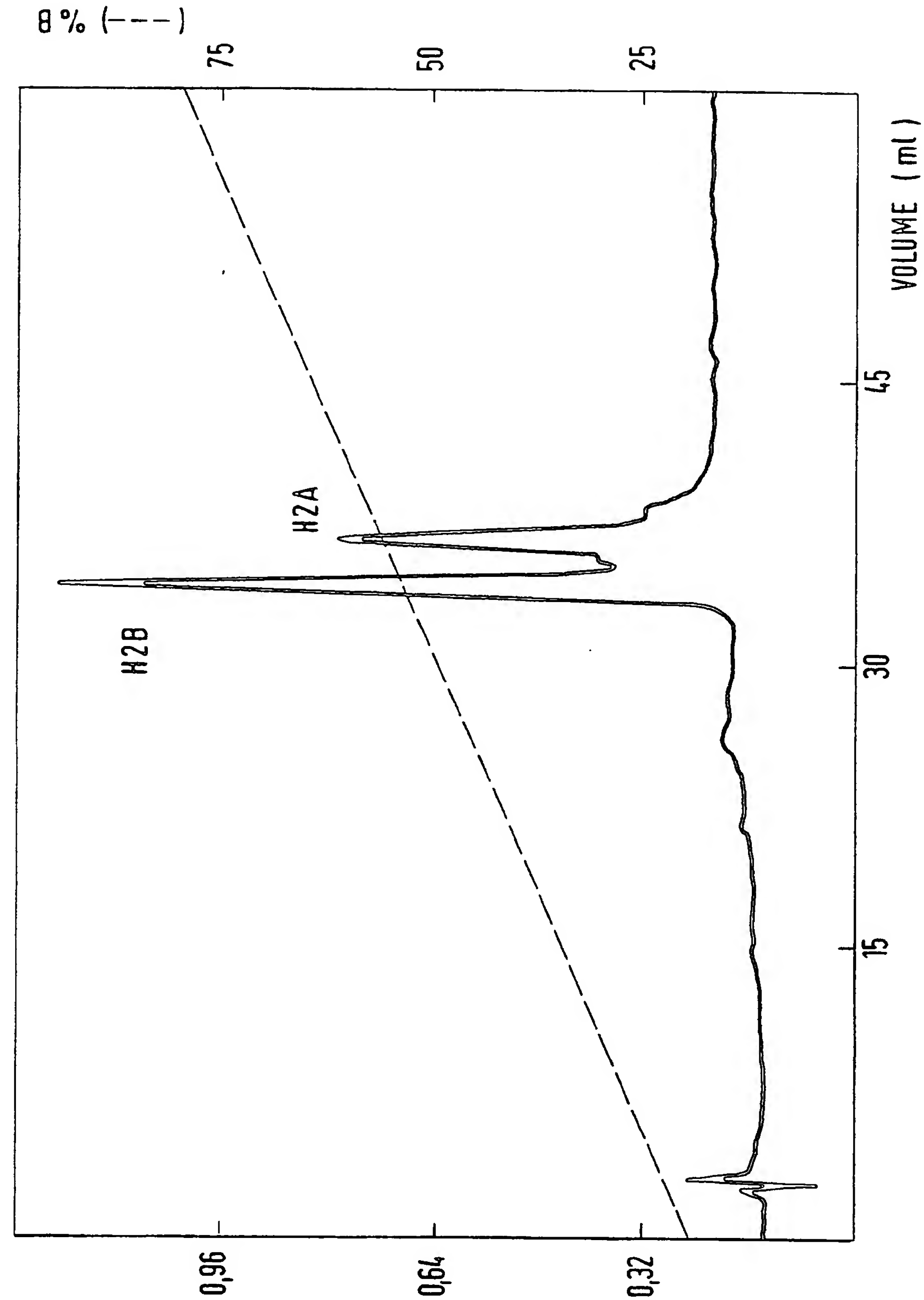

FIG. 1

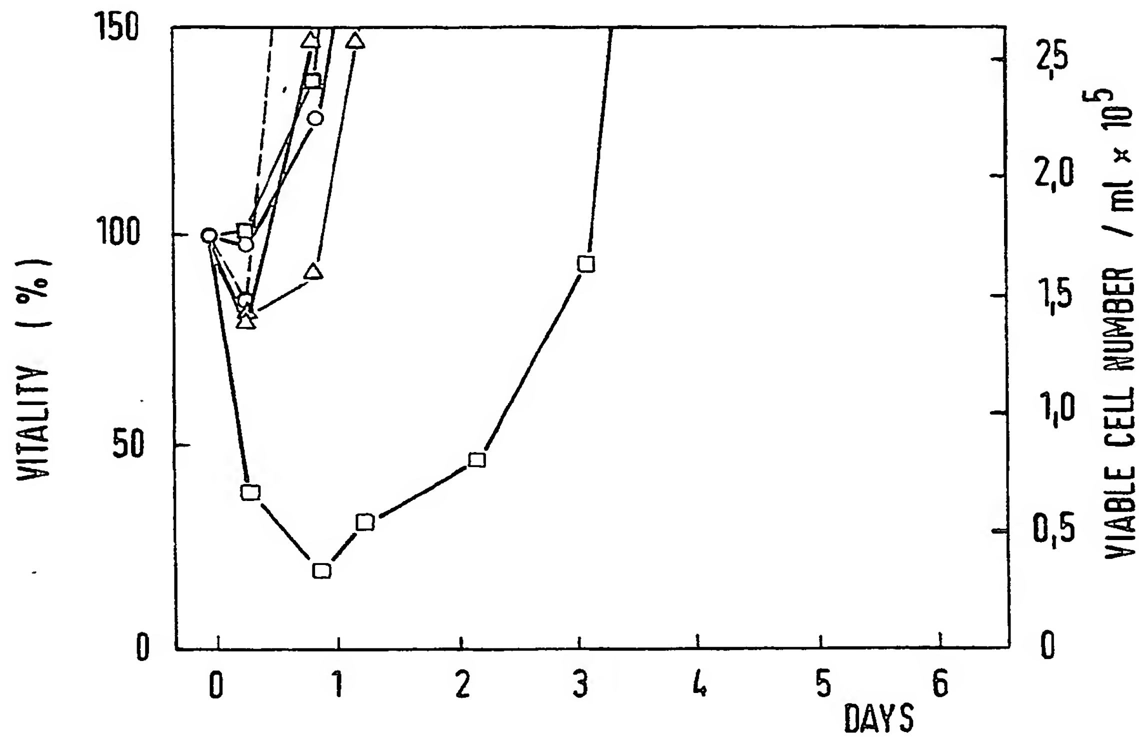

FIG. 2 HTH - CONCENTRATION

△——△ 50 µg/ml
□——□ 100 µg/ml
○——○ 125 µg/ml
△——△ 150 µg/ml
□——□ 200 µg/ml
○- - -○ CONTROL

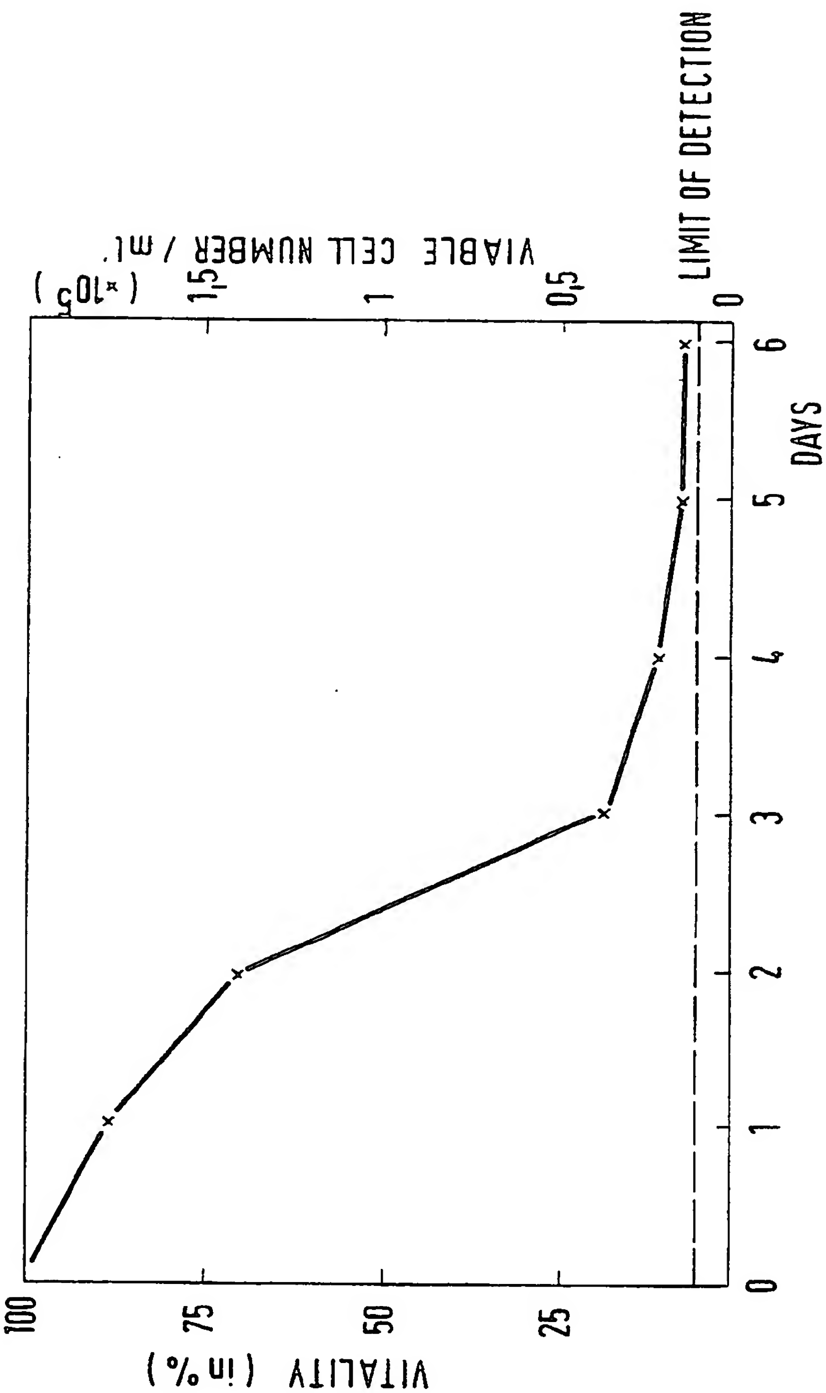
VITALITY (in %)
100
75
50
25
0
1
2
3
4
5
6
DAYS
VIABLE CELL NUMBER / ml
(×10^5)
1,5
1
0,5
0
LIMIT OF DETECTION

FIG. 3

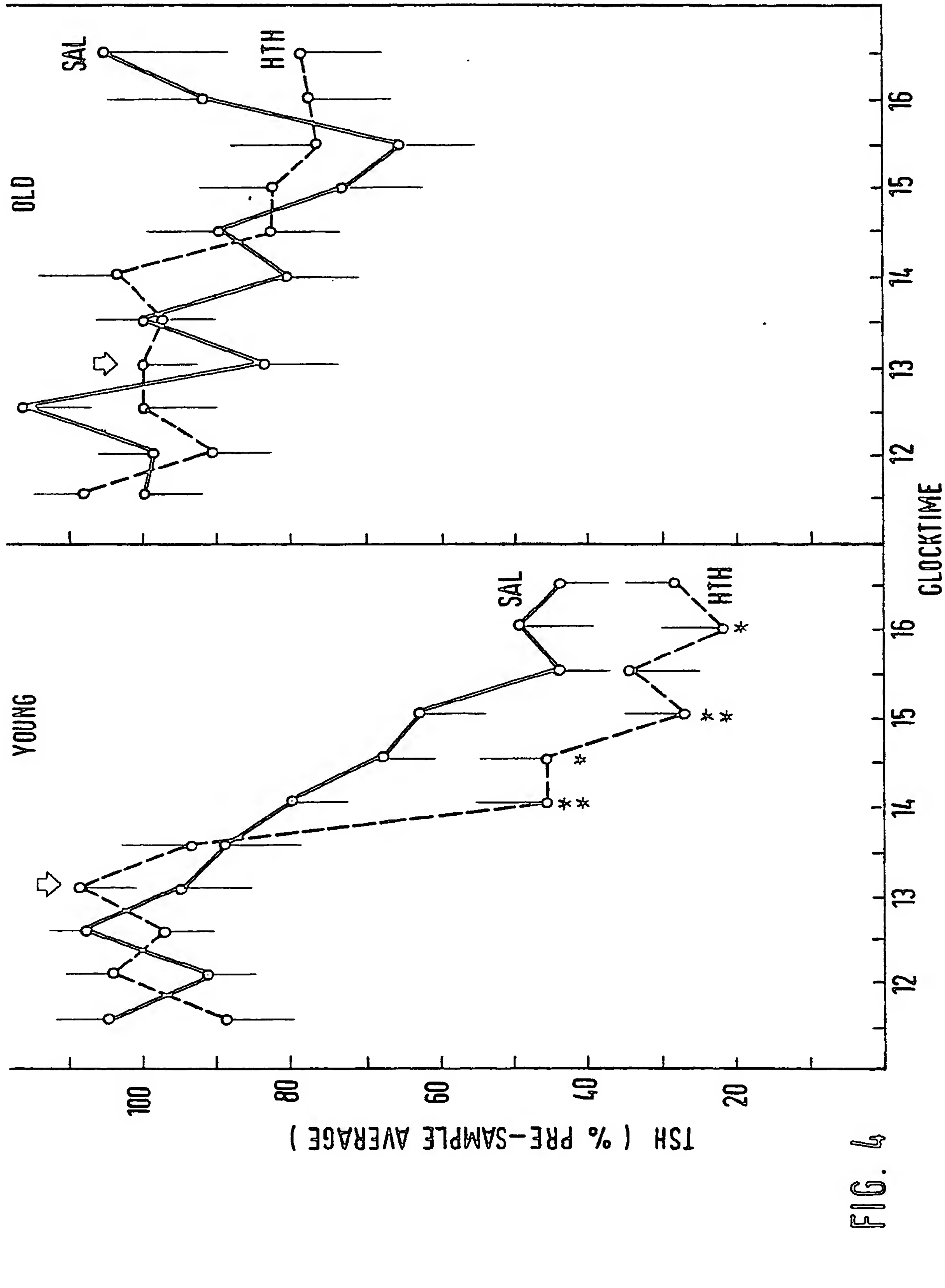
YOUNG
OLD
SAL
HTH
SAL
HTH
TSH (% PRE-SAMPLE AVERAGE)
100
80
60
40
20
12
13
14
15
16
CLOCKTIME

FIG. 4

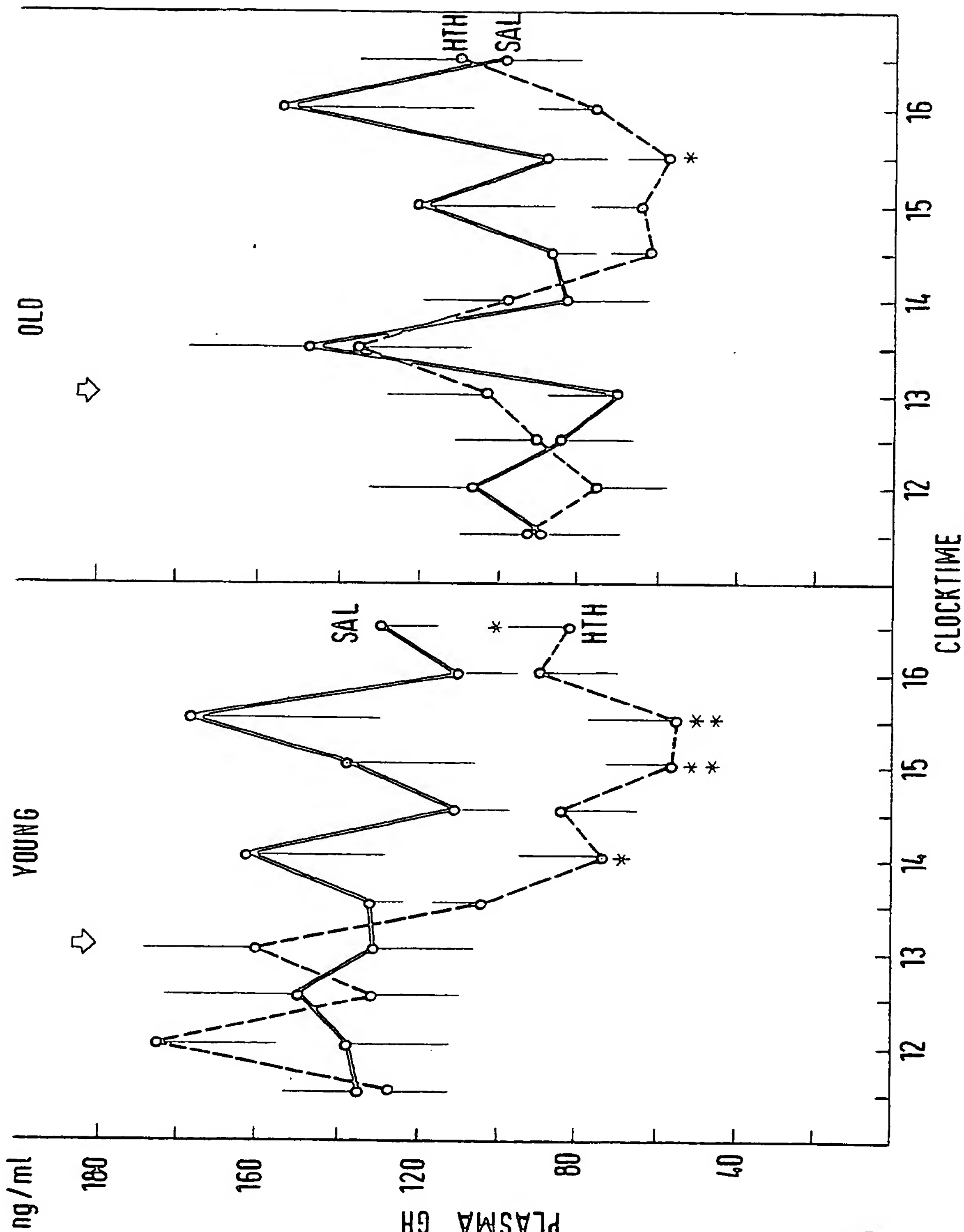
YOUNG
OLD
SAL
HTH
HTH
SAL
CLOCKTIME
12
13
14
15
16
PLASMA GH
ng/ml
180
160
120
80
40

FIG. 5

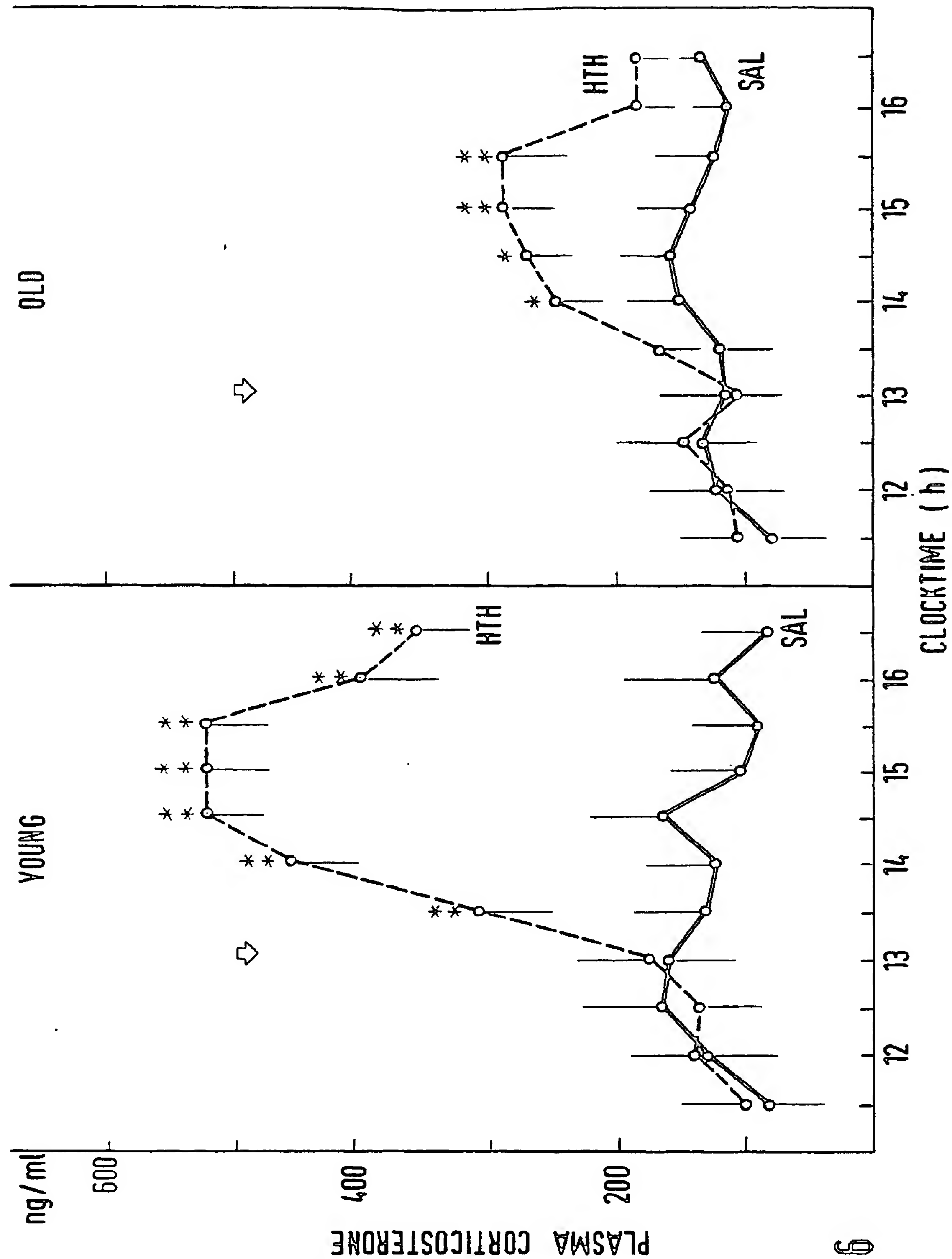
YOUNG
OLD
HTH
SAL
ng/ml
600
400
200
PLASMA CORTICOSTERONE
12
13
14
15
16
CLOCKTIME (h)

FIG. 6

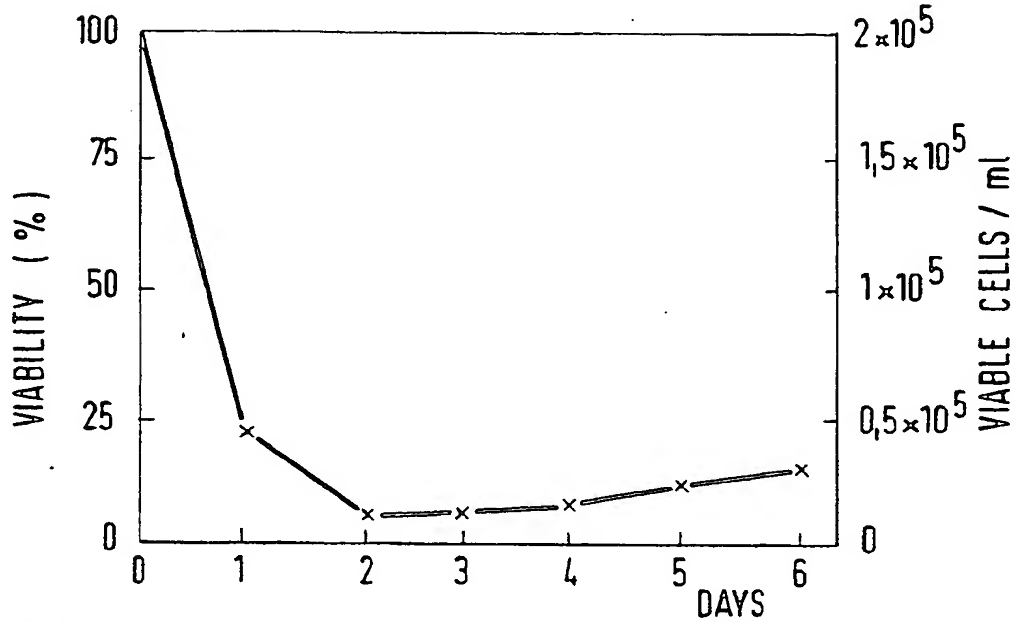

FIG. 7 CANCER CELL LINE DAUDI
(BURKITT-LYMPHOMA)
H1-CONCENTRATION : 250 μg / ml

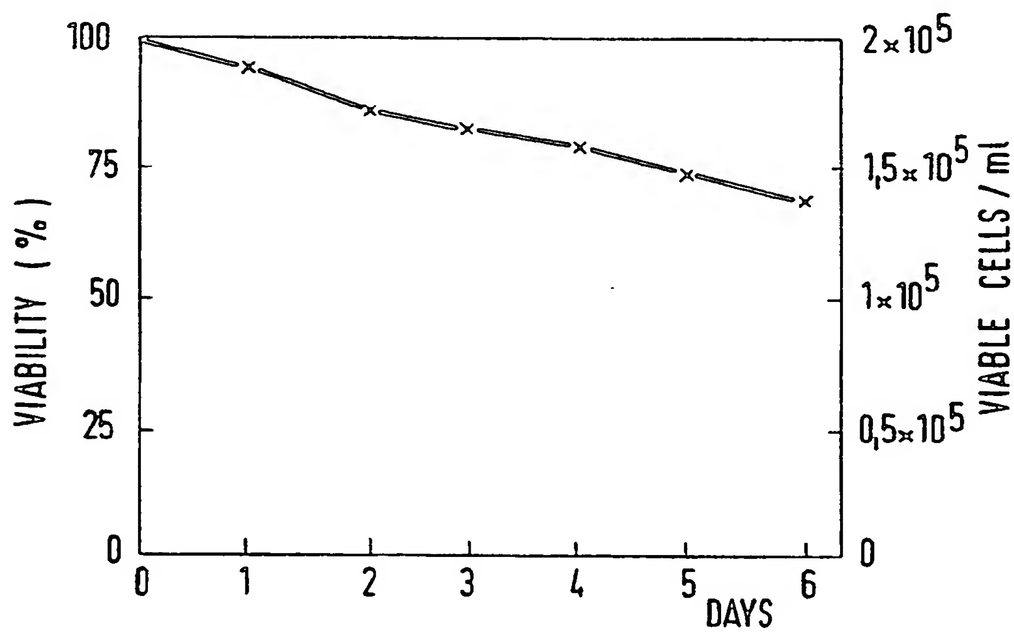

FIG. 8 CANCER CELL LINE EB 2
(BURKITT-LYMPHOMA)
H1-CONCENTRATION : 250 ug / ml

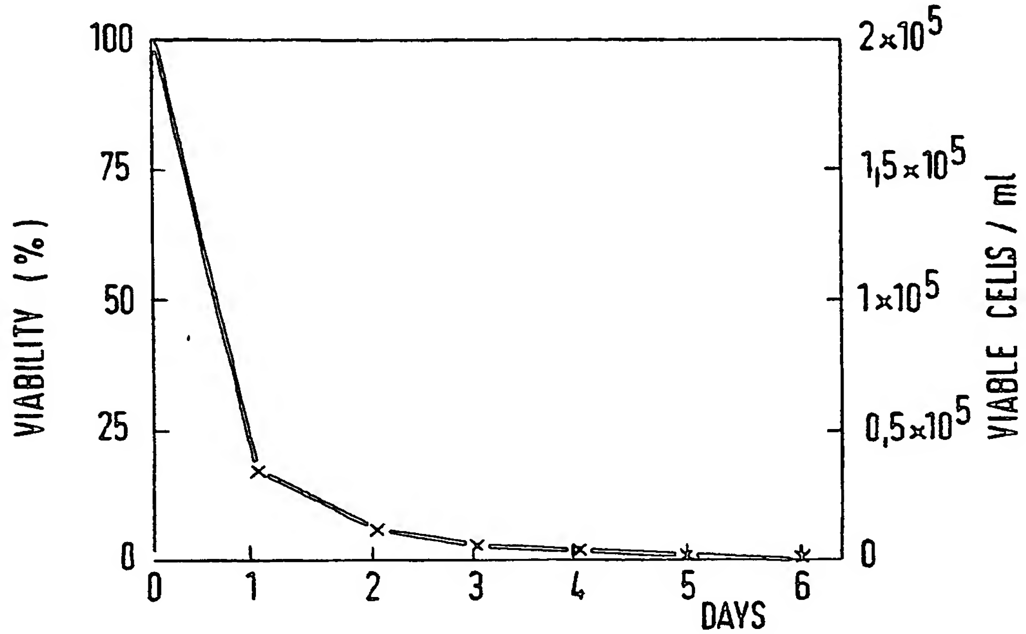

FIG. 9 CANCER CELL LINE IM 9
(MULTIPLE MYELOMA)
H1 - CONCENTRATION : 180 μg / ml

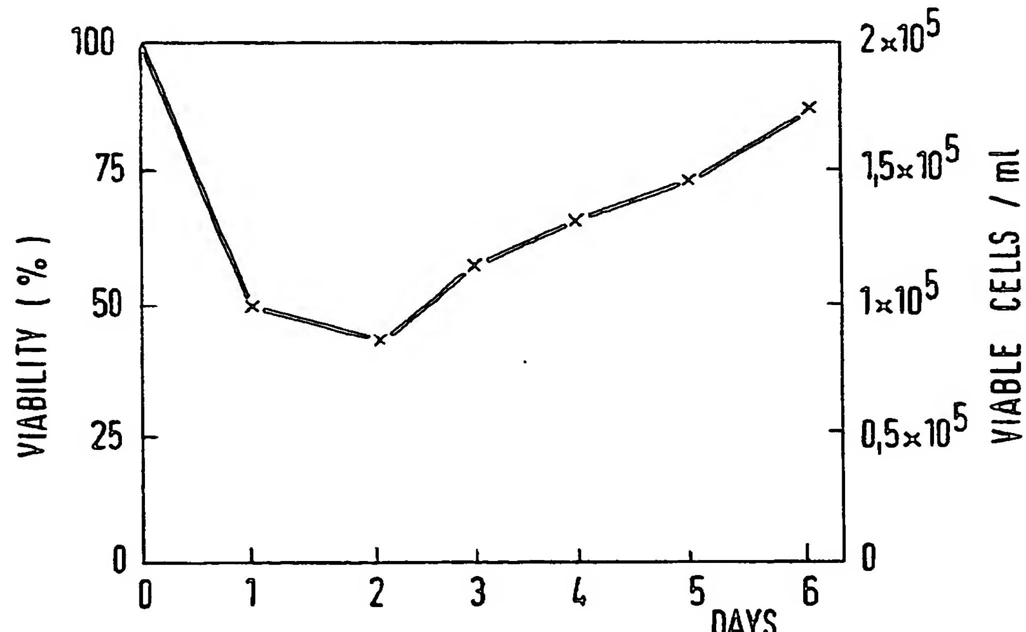

FIG. 10 CANCER CELL LINE CCRF SB
(ACUTE LYMPHOBLASTIC LEUKEMIA)
H1 - CONCENTRATION : 250 μg / ml

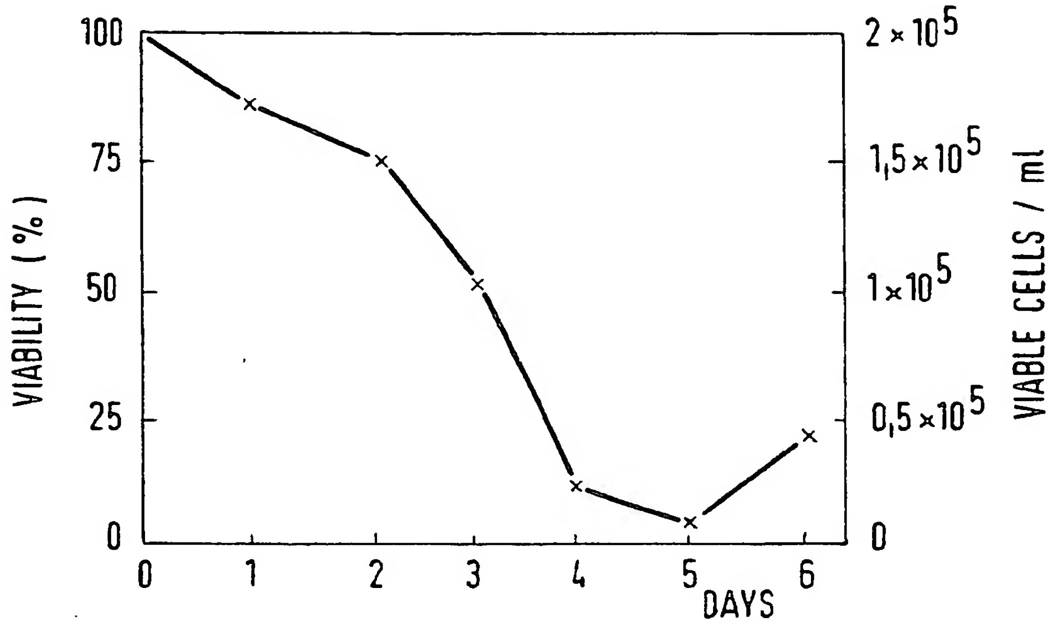

FIG. 11 CANCER CELL LINE CCRF CEM
(ACUTE LYMPHOBLASTIC : LEUKEMIA)
H1 - CONCENTRATION : 250 μg / ml

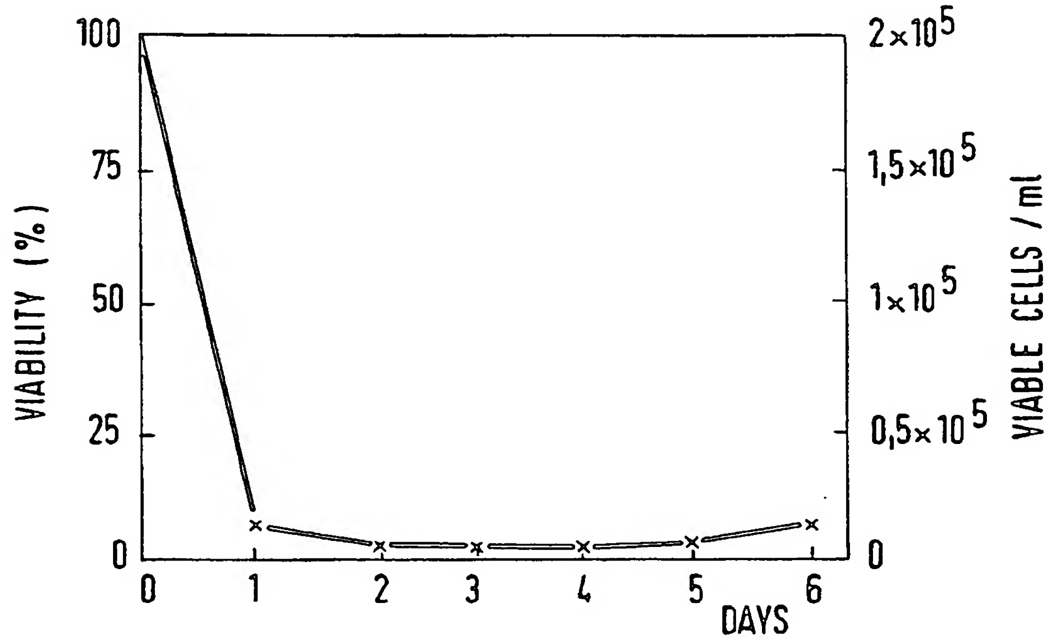

FIG. 12 CANCER CELL LINE NAMALWA
(BURKITT- LYMPHOMA)
H1 - CONCENTRATION : 250 μg / ml

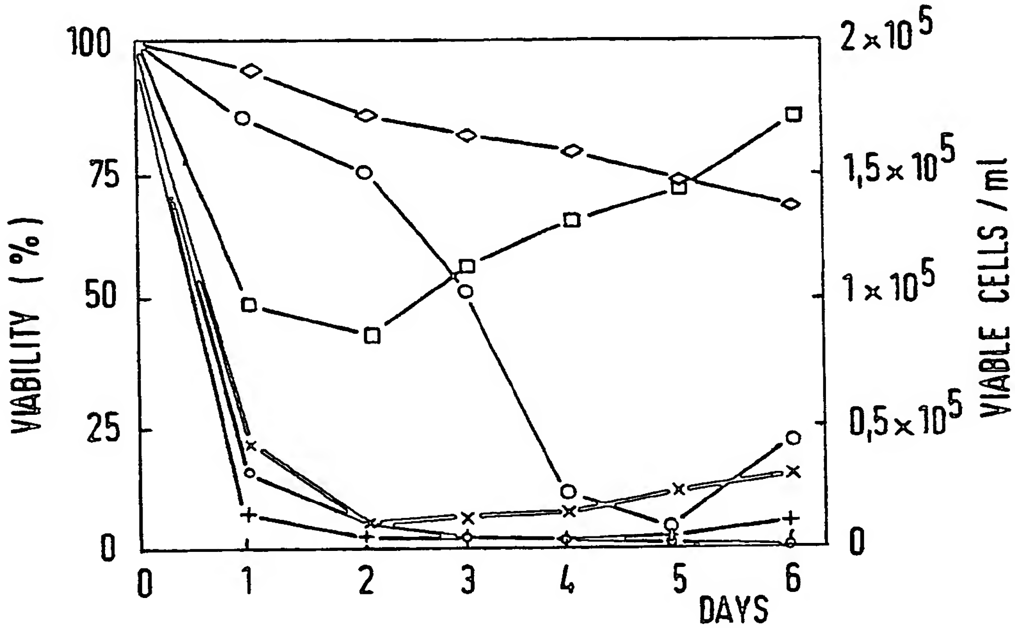

FIG. 13 SUMMERY OF THE FIGURES 7 – 12
H1 – CONCENTRATIONS : 250 μg / ml
(LMO 180 μg / ml)

×——× DAUDI

◇——◇ EB 2

∘——∘ IM 9

□——□ CCRF SB

○——○ CCRF CEM

+——+ NAMALWA

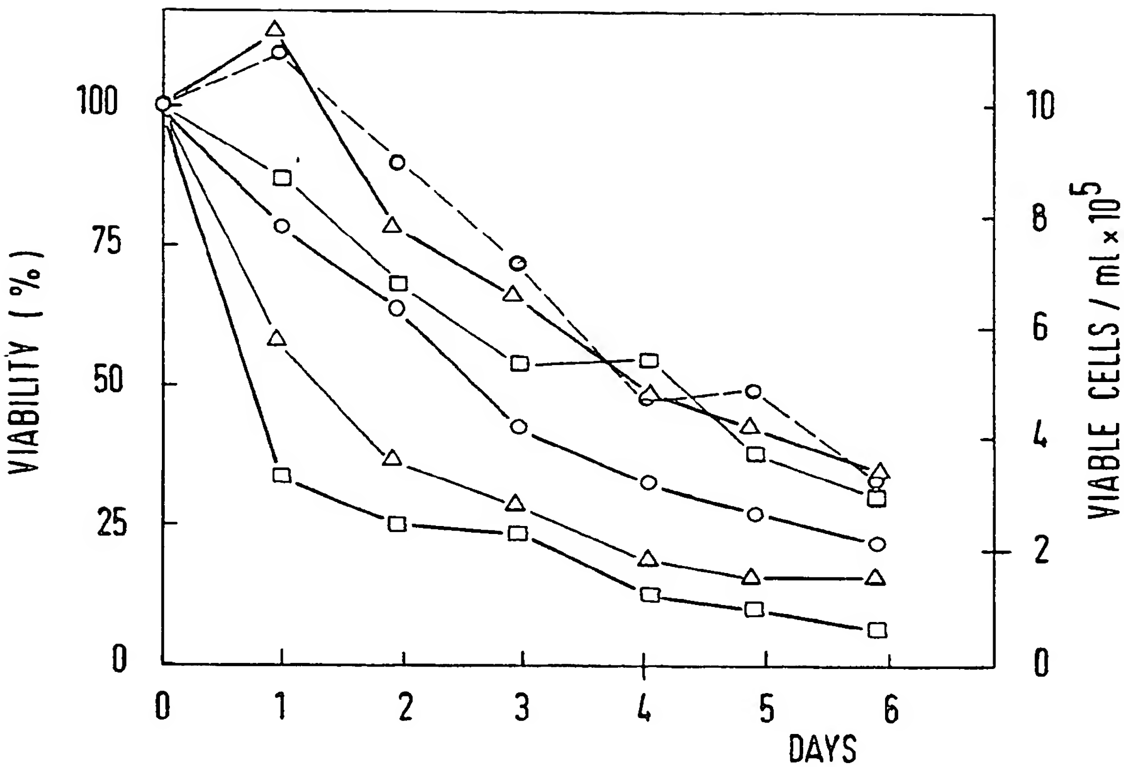

FIG. 14 VIABILITY OF NORMAL MURINE SPLEEN CELLS AS A FUNKTION OF H1- CONCENTRATION

△——△ 50 µg/ml
□——□ 100 µg/ml
○——○ 125 µg/ml
△——△ 150 µg/ml
□——□ 200 µg/ml
○- - -○ CONTROL

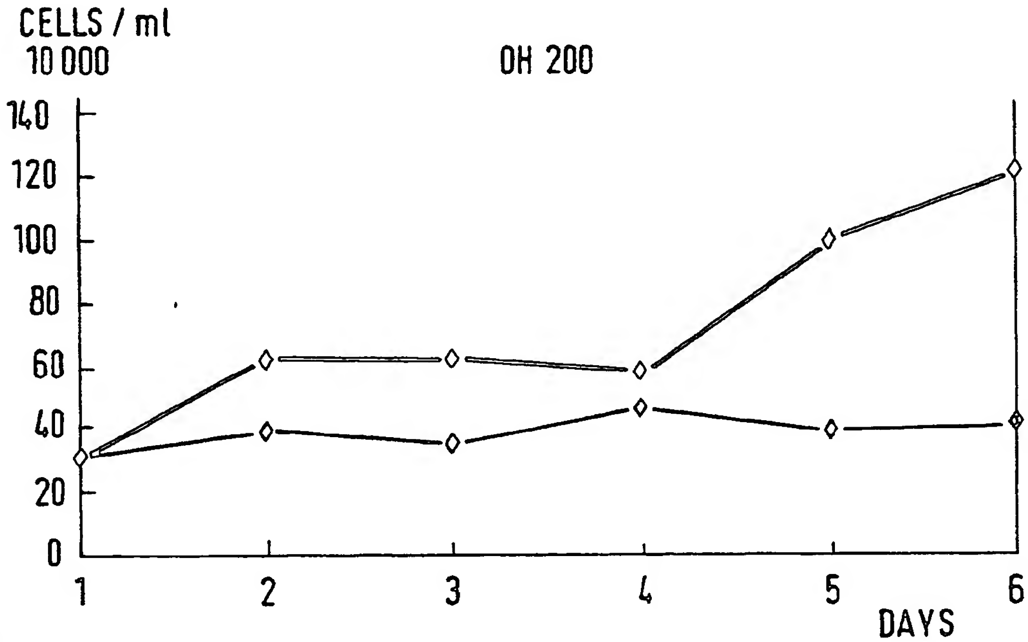
CELLS / ml
10 000
OH 200
140
120
100
80
60
40
20
0
1
2
3
4
5
6
DAYS

FIG. 15

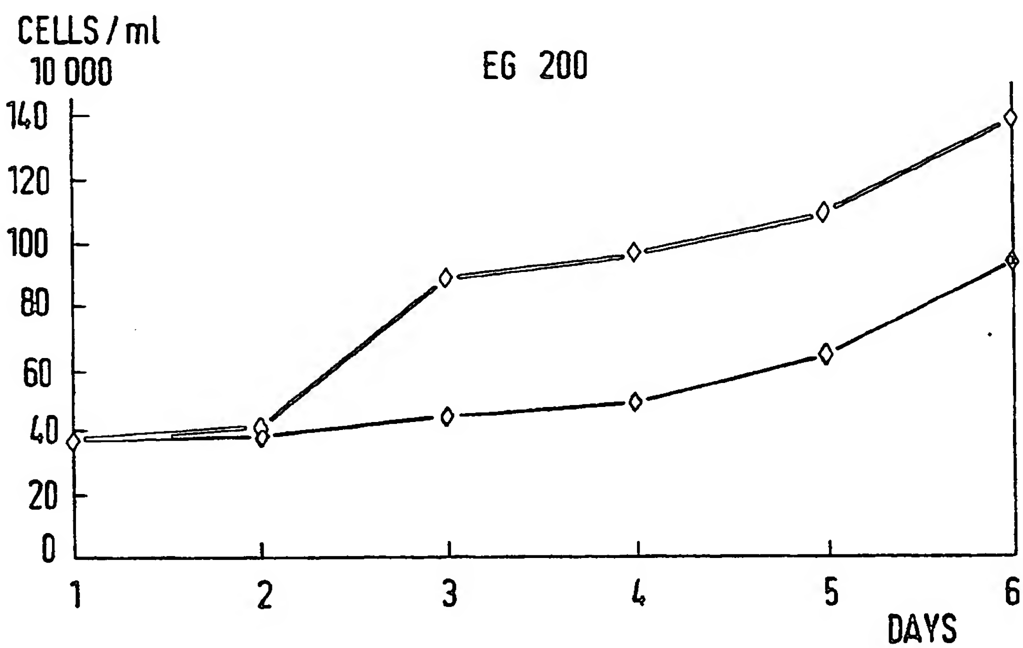
CELLS / ml
10 000
EG 200
140
120
100
80
60
40
20
0
1
2
3
4
5
6
DAYS

FIG. 16

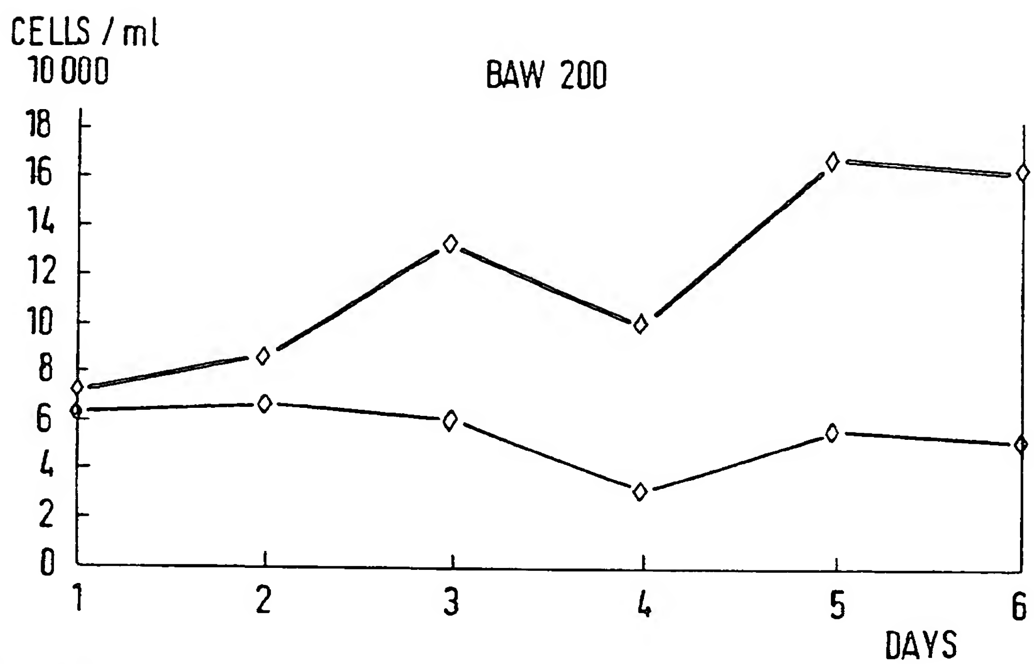

FIG. 17

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 10 6311

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	PROC. NATL. ACAD. SCI. USA, vol. 82, no. 15, August 1985, pages 4871-4875, Washington, DC, US; R. REICHHART et al.: "Preparations of homeostatic thymus hormone consist predominantly of histones 2A and 2B and suggest additional histone functions" * Page 4871, left-hand column, lines 27-41; page 4873, left-hand column, lines 22-31; page 4875, left-hand column, lines 22 - end *	1-13	A 61 K 37/24 A 61 K 37/02
D,A	EP-A-0 149 468 (V. RUSCH) * The claims; page 14, lines 14-19 *	1-13	
A	BIOLOGICAL ABSTRACTS, vol. 75, no. 1, 1983, page 339, abstract no. 3258, Biological Abstracts, Inc., Philadelphia, PA, US; V.G. MOROZOV et al.: "Isolation, purification and identification of an immunodulating polypeptide from human and calf thymus", & BIOKHIMIYA 46(9): 1652-1659, 1981 * The entire abstract *	1,4,10, 13	TECHNICAL FIELDS SEARCHED (Int. Cl.5) A 61 K
A	CHEMICAL ABSTRACTS, vol. 110, no. 7, 13th February 1989, page 533, abstract 55827v, Columbus, Ohio, US; & JP-A-63 139 134 (KYOWA HAKKO KOGYO CO., LTD) 10-06-1988 * The entire abstract *	2-4,10, 13	
P,X	DE-A-3 737 274 (V. RUSCH) * The claims; page 2, lines 31-38,45-48 *	2-13	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	11-07-1990	RYCKEBOSCH A.O.A.

CATEGORY OF CITED DOCUMENTS

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